AMENDMENTS TO THE SPECIFICATION

Page 3, lines 7-12:

In one aspect, this invention is directed to a porcine adipocyte polypeptide (i.e., the porcine leptin protein) substantially free of other porcine polypeptides, or functional derivatives thereof. The present invention includes a porcine adipocyte polypeptide of at least about 8 amino acids of the amino acid sequence depicted in FIGS. 1A-1D ~~(SEQ. ID NO. 1)~~, preferably the amino acid sequence depicted in FIG. 2 ~~(SEQ. ID NO. 2)~~ (SEQ ID NO: 3), still more preferably, the amino acid sequence depicted in FIG. 3 ~~(SEQ. ID NO: 3 and SEQ. ID NO. 4)~~ (SEQ ID NO:5), or functional derivatives thereof.

Page 3, line 13, through page 4, line 2:

The present invention is also directed to a single or double stranded DNA or an RNA molecule (and their respective allelic variants) consisting essentially of a nucleotide sequence that encodes the above polypeptide, the DNA or RNA molecule being substantially free of other porcine DNA or RNA sequences or, in other words, isolated or an isolate. The DNA molecule is preferably a single or double stranded DNA molecule having a nucleotide sequence consisting essentially of at least about 20 nucleotides of the nucleotide sequence depicted in FIGS. 1A-1D (SEQ. ID NO: 1), preferably, the nucleotide sequence depicted in FIG.2 (SEQ ID NO: 2), still more preferably the nucleotide sequence depicted in FIG. 3 ~~(SEQ. ID NO: 3)~~ (SEQ ID NO: 4), or a sequence complementary to the nucleotide sequences depicted in FIGS. 1A-3 (SEQ. ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 4 ~~and SEQ. ID NO. 3~~), or an allelic variant thereof substantially free of other porcine DNA sequences. The RNA molecule is preferably an mRNA sequence encoding the above porcine adipocyte polypeptide, or functional derivatives thereof.

Page 5, lines 19-20:

FIGS. 1A-1D depicts the nucleotide sequence of the porcine leptin gene and the amino acid translation of the porcine leptin coding sequences (SEQ. ID NO: 1, ~~and~~ SEQ ID NO: 2, and SEQ ID NO: 3).

Page 5, line 20, through page 6, line 1:

FIG. 2 depicts the nucleotide sequence and the amino acid translation of the coding region of the entire porcine leptin cDNA (i.e., signal peptide and secreted protein) (SEQ ID NO: 2 and SEQ ID NO: 3 ~~SEQ. ID NO. 1 and SEQ. ID NO. 2~~).

Page 6, lines 2-3:

FIG. 3 depicts the nucleotide sequence and the amino acid translation of the porcine leptin cDNA corresponding to the secreted porcine leptin protein (SEQ ID NO: 4 and SEQ ID NO: 5).

Page 6, lines 4-6:

FIG. 4 shows a comparison of the porcine leptin cDNA sequence corresponding to the entire porcine leptin cDNA ~~protein~~ (SEQ. ID NO. 1) with the murine (SEQ ID NO: 8 ~~SEQ. ID NO. 6~~) and human (SEQ ID NO: 7 ~~SEQ. ID NO. 5~~) sequences.

Page 7, line 15, through page 8, line 1:

The polypeptide of this invention has an amino acid sequence as depicted in FIGS. 1A-1D and 2 (SEQ ID NO: 3) ~~SEQ. ID NO. 1 and SEQ. ID NO. 2~~), and preferably as depicted in FIG. 3 ~~(SEQ. ID NO. 3 and SEQ. ID NO. 4)~~ (SEQ ID NO: 5). Also intended within the scope of the present invention is any polypeptide having at least about 8 amino acids present in the above-mentioned sequence. Sequences of this length are useful as antigens and for making immunogenic conjugates with carriers for the production of antibodies specific for various epitopes of the entire protein. Such polypeptides are also useful in screening such antibodies and in the methods of the present invention directed to detection of the leptin protein in biological samples. It is well-known in the art that polypeptides of about 8 amino acids are useful in generation of antibodies to larger proteins of biological interest.

Page 10, line 14, through page 11, line 1:

The polypeptide of the present invention is encoded by a nucleic acid molecule, one strand of which has the nucleotide sequence shown in FIGS. 1A-1D (SEQ. ID NO. 1), preferably as shown in FIG. 2 (SEQ. ID NO. 2), and still more preferably as shown in FIG. 3 (SEQ ID NO: 4 ~~SEQ. ID NO. 3~~). The present invention is directed to a DNA sequence encoding the polypeptide, or a functional derivative thereof, substantially free of other porcine DNA sequences. Such DNA may be single stranded (i.e., sense strand, antisense strand or cDNA sequence) or double stranded. The DNA sequence should preferably have about 20 or more nucleotides to allow hybridization to another polynucleotide. In order to achieve higher specificity of hybridization, characterized by the absence of hybridization to sequences other than those encoding the polypeptide or a functional derivative thereof, a length of at least about 50 nucleotides is preferred.

Page 11, lines 2-13:

The present invention is also directed to an RNA molecule (or an allelic variant thereof) comprising a mRNA sequence encoding the polypeptide of this invention, or a functional derivative thereof, and the antisense RNA (or a fragment thereof) of the mRNA. The antisense RNA is, of course, simply the complement to the cDNA sequence (cDNA corresponds to mRNA except uracil replaces thymidine; cDNA and mRNA as "sense", so the complements of these molecules are "antisense"). Antisense RNA (or antisense "oligonucleotides") are described more fully in Molecular Biology and Biotechnology, Antisense Oligonucleotides, Structure and Function of, Uhlmann and Peyman, pp. 38-45 (Wiley-VCH, 1995). The antisense RNA of this invention is the complement, or a fragment, of the nucleotide sequence shown in FIGS. 1A-1D ~~and 2~~, FIG. 2 and FIG. 3 (SEQ ID NO: 1, SEQ ID NO: 2 and SEQ ID NO: 4), or an allelic variant thereof. If a fragment, the antisense RNA sequence should preferably have about 20, more preferably about 50 or more, nucleotides to allow binding to a complementary region of mRNA sufficient to inhibit protein biosynthesis.

Page 22, lines 4-9:

Sense strand:

5'-GGATCCGGTCTCAGGCCGTGCC(C/T)ATCCA(A/G)AAAGTCC-3' (SEQ ID NO: 8 ~~SEQ ID NO:7~~).

Antisense strand:

5'-GAATTCAGCGCTGCA(C/T)(C/T)CAGGGCT(G/A)A(G/C)(G/A)TC-3' (~~SEQ ID NO:8~~ SEQ ID NO: 9)

Page 23, lines 2-8:

The nucleotide sequence of the porcine leptin gene comprising 5917 base pairs, and the amino acid translation of the leptin coding sequences are depicted in FIGS. 1A-1D (SEQ. ID NO. 1). The nucleotide sequence and the amino acid sequence of the entire porcine leptin cDNA (i.e., signal peptide and secreted proteins) comprising 501 base pairs and 166 amino acids ~~167 amino acids~~ are depicted in FIG. 2 (SEQ ID NO: 2 and SEQ ID NO: 3 ~~SEQ. ID NO. 1 and SEQ. ID NO. 2~~). The nucleotide sequence and the amino acid sequence of the porcine leptin cDNA corresponding to the secreted protein alone and comprising 435 base pairs and 145 amino acids are depicted in FIG. 3 (SEQ ID NO: 4 and SEQ ID NO: 5 ~~SEQ. ID NO. 3 and SEQ. ID NO. 4~~).

Page 23, lines 9-11:

There was an 83% identity between the pig and human (SEQ ID NO: 6 ~~SEQ. ID NO. 5~~) cDNA sequence and a 76% identity between the pig (SEQ. ID NO. 1) and mouse (SEQ ID NO: 7 ~~SEQ. ID NO. 6~~) cDNA sequence as depicted in FIG. 4.

Page 25, lines 7-15:

The 5917 bp Hind III fragment was subcloned into Bluescript II SK+ (Stratagene, Inc.). Both strands of the sequence was determined using progressive nested deletions using Exonuclease III and Mung Bean nuclease. Sequencing reactions were carried out with Sequenase V2.0. This sequence was 5917 bp in length and contains the entire coding region in two exons (Fig. 1, SEQ. ID NO. 1). There was 78.6% nucleotide identity between the pig and human as well as 71.2% nucleotide identity between pig and mouse coding sequences. The splice junctions for the two exons were confirmed by the cDNA sequence. The cDNA sequence of the protein coding region is shown in FIG.2 (~~SEQ. ID NO. 1 and SEQ. ID NO. 2~~ SEQ ID NO:2 and SEQ ID NO:3). The 501 bp sequences encodes 166 amino acid residue leptin polypeptide with a predicted molecular mass of 18,334 Da.

Page 29, line 3, through page 30, line 3:

A porcine ob (obese gene) cDNA probe was amplified from adipose tissue mRNA using the reverse transcriptase-polymerase chain reaction (RT-PCR). First strand cDNA synthesis reactions were carried out using 1-2 μg of porcine adipose tissue total RNA, 150 pmol of random hexamer oligonucleotides, 500 nM dNTP, 200μl of Superscript II reverse transcriptase (LifeTechnologies, Inc., Bethesda, Md., USA) in 20 μl of the supplied buffer. The reactions were incubated for 1 h at 37°C. and terminated by heating to 70°C. for 10 min. The ob cDNA product was amplified by PCR using the following degenerate primers with restriction site linkers for Bam/HI and XbaI respectively; sense strand 5'-GTGCCYATCCARAAAGTCC-3' (SEQ ID NO: 8) and antisense strand 5'-GCAYYCAGGGCTRASRTC-3' (SEQ ID NO: 9). Adipose tissue cDNA was added as template to 50 μl PCR reactions made in the manufacturer's buffer with 100 pmol of each primer and 2.5 U of Taq DNA polymerase (LifeTechnologies, Inc.). A three stage amplification was carried out under the following conditions; Stage 1-95°C, 3 min; 52°C, 1 min; 72°C, 1 min; 1 cycle; Stage 2-94°C, 45s; 52°C, 45s; 72°C, 1 min; 4 cycles; Stage 3-94°C, 45s; 55°C, 30s; 72°C, 1 min; 28 cycles. The PCR products were digested with the restriction enzymes BamHI and XbaI and purified by electrophoresis in 1% NuSieve low melting point agarose (FMC Bioproducts, Rockland, ME, USA). The *ob* cDNA was ligated into Bluescript II SK + (Stratagene Inc. LaJolla, CA, USA) and transformed into MCR DH5α (Life Technologies, Inc.) And plated on LB plates containing 50 μg/ml ampicillin for plasmid selection. Twelve *E. coli* colonies were isolated that contained the porcine *ob* cDNA and plasmid DNA was isolated for

sequencing. Dideoxy sequencing reactions were carried out using [^{35}S] dATP labeling with Sequence V2.0. The sequence samples were loaded on 5% Long Ranger (FMC Bioproducts) for denaturing gel electrophoresis according to the manufacturer's recommendations.